Learning capabilities and CA1-prefrontal synaptic plasticity in a mice model of accelerated senescence

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Abstract

SAMP8 mice represent a suitable model of accelerated senescence as compared with SAMR1 animals presenting normal aging. Five-month-old SAMP8 mice presented reflex eyelid responses like those of SAMR1 controls, but were incapable of acquiring classically-conditioned eye blink responses in a trace (230 milliseconds [ms] of interstimulus interval) paradigm. Although SAMP8 mice presented a normal paired-pulse facilitation of the hippocampal CA1-medial prefrontal synapse, an input/output curve study revealed smaller field excitatory postsynaptic potentials (fEPSPs) in response to strong stimulations of the CA1-prefrontal pathway. Moreover, SAMP8 mice did not show any activity-dependent potentiation of the CA1-prefrontal synapse across the successive conditioning sessions shown by SAMR1 animals. In addition, SAMP8 mice presented a functional deficit during an object recognition test, continuing to explore the familiar object when controls moved to the novel one. Alert behaving SAMP8 mice presented a significant deficit in long-term potentiation (LTP) at the CA1-medial prefrontal synapse. According to the present results, SAMP8 mice present noticeable functional deficits in hippocampal and prefrontal cortical circuits directly related with the acquisition and storage of new motor and cognitive abilities.

Keywords: Aging; Classical eyelid conditioning; Object recognition; SAMP8; SAMR1; LTP; Activity-dependent synaptic plasticity

1. Introduction

The study of learning and memory deficits that are pathognomonic of the aging process requires the availability of suitable animal models. In this regard, senescence-accelerated-prone 8 (SAMP8) mouse strain and senescence-accelerated-resistant 1 (SAMR1) mouse strain are characterized by an accelerated senescence and a normal aging respectively (Takeda, 2009). In fact, this spontaneous model of early aging presents some advantages over genetically manipulated models related to the aging process. SAMP8 is a mouse strain that displays generalized accelerated aging (Takeda et al., 1997) presenting cognitive deficiencies that could be related to the accumulation of amyloid aggregates, oxidative stress, and other brain anomalies similar to those observed in Alzheimer’s disease (Butterfield and Poon, 2005; Carretero et al., 2009; Del Valle et al., 2010; Díez-Vives et al., 2009; Umezawa et al., 2003). It has already been reported that SAMP8 animals present evident deficits in selective behavioral and learning tasks, suggesting that they can be used as an experimental model of geriatric disorders (Flood and Morley, 1993; Markowska et al., 1998; Miyamoto et al., 1986; Morley et al., 2000; Nomura et al., 1997; Zheng et al., 2009). Indeed, many of the pathological phenotypes described in SAMP8 mice can be associated to age-related pathologies and/or subsequent to physiological senescence (Cotran et al., 1989; Takeda, 2009). The senescence score in SAMP strains is about twice that in SAMR mice (7.97 against 3.94), while the median survival time is
Animals were allowed ad libitum access to commercial constant temperature (21°C) and humidity (50%) and were kept in collective cages on a 12-hour light/dark cycle with electrical interferences. The EMG activity of the orbicularis oculi muscle was recorded using differential amplifiers with a bandwidth of 1 Hz to 10 kHz (Grass Technologies, West Warwick, RI, USA). Field EPSP recordings were also carried out during the light cycle and according to European Union Council (2003/65/EU) and Spanish (BOE 252/34367-91, 2005) guidelines for the use of laboratory animals in chronic electrophysiological and behavioral studies. All experimental protocols were also approved by the Ethics Committee of the Pablo de Olavide University.

2.2. Surgical procedures

Under deep anesthesia (ketamine 35 mg/kg, and xylazine 2 mg/kg intraperitoneally), animals were implanted with 4 electrodes in the upper eyelid of the left eye (Fig. 1A). Electrodes were made of Teflon-insulated, annealed stainless steel wire (50 μm in diameter, A-M Systems, Carlsborg, WA, USA). One pair of electrodes was aimed at the supraorbital nerve, and served for the presentation of electrical stimuli. The second pair of electrodes was implanted in the ipsilateral orbicularis oculi muscle to record its EMG activity. Electrode tips were bent as a hook to facilitate a stable insertion in the upper eyelid.

Animals for classical eye blink conditioning were also implanted with bipolar stimulating electrodes aimed at the right CA1 area of the dorsal hippocampus (2.75 mm lateral and 3.16 mm posterior to bregma; depth from brain surface, 2.0–2.25 mm; Paxinos and Franklin, 2001) and with a recording electrode aimed at the right infralimbic area of the medial prefrontal cortex (0.3 mm lateral and 1.94 mm anterior to bregma; depth from brain surface, 3.0–3.3 mm). These electrodes were made of 50 μm, Teflon-coated tungsten wire (Advent Research Materials Ltd, Eynsham, England). The final position of medial prefrontal recording electrodes (Fig. 1B and D) was determined using as a guide the field potential depth profile evoked by paired (20 ms interval) pulses presented in the ipsilateral CA1 area.

Animals for the LTP experiment were implanted with stimulating and recording electrodes only at the hippocampal CA1 and medial prefrontal cortex sites indicated above.

A 0.1 mm bare silver wire was affixed to the skull as a ground. Wires were connected to 1 (for LTP) or 2 (for classical conditioning) 4-pin sockets (RS-Amidata, Madrid, Spain). Sockets were fixed to the skull with the help of 2 small screws and dental cement (Domínguez-del-Toro et al., 2004; Gruart et al., 2006). After surgery, animals were kept in individual cages, with free access to food and water, for the rest of the experiment. Recording sessions started 1 week after surgery.

2.3. Recording and stimulating procedures

During recording sessions, animals were placed individually in a plastic chamber (5 cm × 15 cm × 15 cm), inside a larger Faraday box (30 cm × 30 cm × 20 cm) to eliminate electrical interferences. The EMG activity of the orbicularis oculi muscle was recorded using differential amplifiers with a bandwidth of 1 Hz to 10 kHz (Grass Technologies, West Warwick, RI, USA). Field EPSP recordings were also car-
ried out using Grass P511 differential amplifiers with the help of a high-impedance probe ($2 \times 10^{12} \, \Omega$, 10 picofarads [pF]).

For input/output curves (Fig. 2A–C), animals ($n = 15$) were stimulated in the CA1 area with paired pulses (40 ms of interstimulus interval) at increasing intensities (0.3–4.8 mA). We also checked the effects of paired pulses at different (10, 20, 40, 100, 200, and 500 ms) interstimulus intervals when using intensities corresponding to 40% of the amount necessary to evoke a saturating response (Fig. 2D).

A complete study of the mixed effects of increasing intensities within the range of interstimulus intervals indicated above was also carried out in 5 selected animals (Fig. 3). In all cases, the pair of pulses of a given intensity was repeated 5 times with time intervals of 30 seconds (s), to avoid as much as possible interferences with slower short-term potentiation (augmentation) or depression processes (Zucker and Regehr, 2002). In order to avoid any cumulative effects, intensities and intervals were presented at random (Madroñal et al., 2009).

2.4. Classical conditioning procedures

The classical conditioning of eyelid responses was carried out using a trace paradigm ($n = 6$ SAMR1 and $n = 7$ SAMP8 animals; Fig. 4). Animals were presented with a tone (6000 Hz, 70 dB, 20 ms) as a CS, followed 250 ms from CS onset by an electrical stimulation (500 μs, 3 × threshold) as a US. Intervals between paired CS-US presentations were separated at random by 30 ± 5 s. For habituation and extinction sessions, the CS was presented alone, also at intervals of 30 ± 5 s. A total of 1 habituation, 5 conditioning, and 3 extinction sessions (100 trials each) were presented to each animal across 9 successive days. Only the first 50 trials of each session were analyzed (Gruart et al., 2006; López-Ramos et al., 2007).

Synaptic field potentials were evoked in the infralimbic area of the medial prefrontal cortex during habituation, conditioning, and extinction sessions by single 50 μs, square, biphasic (negative-positive) pulses applied to the CA1 area 125 ms after CS presentation. Stimulus intensities ranged from 0.6 to 1.2 mA. For each animal, the stimulus intensity was set well below the threshold for evoking a population spike, usually 30–40% of the intensity necessary for evoking a maximum fEPSP response (Gruart et al., 2006; Gureviciene et al., 2004). An additional criterion for selecting stimulus intensity was that a second stimulus, presented 40 ms after a conditioning pulse, evoked a larger (> 20%) synaptic field potential (Bliss and Gardner-Medwin, 1973).

2.5. Object recognition task

For the object recognition task, all the animals ($n = 9$ per group) were individually habituated to an open field (40 cm × 25 cm × 15 cm), under low illumination conditions, and with no objects, for 5 minutes. During the training session, 2 unknown but identical objects (O1 and O2) were placed into the open field, and the animals were allowed to explore them freely for 10 minutes. The time spent exploring each object and the total approach time were quantified. After each trial, the apparatus and the objects were thoroughly
cleaned with 70% ethanol to avoid odor recognition. One hour after the first training, mice were allowed to explore the open field for another 10 minutes, when 1 of the 2 familiar objects (O1 or O2) was replaced by an identical object (O3), and the other (O1 or O2) by a novel object (B1). The time spent exploring each object and the total approach time were quantified again. Within each experimental group, the object O1 was replaced by the new object for half of the animals, whereas object O2 was changed for the other half. The aim was to avoid any issue related to spatial preference associated, or not, with the location of the 2 objects. Twenty-four hours after the initial training, the mice were tested again, with a new object (C1) and an object identical to the old one (B2). The same procedure was carried out 72 hours after the initial training (D1 and C2; see Eleore et al., 2007 for details). The attention index (i.e., the percentage of attention) for each object (familiar or new) was expressed as a percentage of the total attention to the 2 objects exhibited during each session (Fig. 5).

2.6. LTP induction

To evoke LTP, animals \((n = 21)\) SAMR1 and \(n = 7)\) SAMP8) were presented with an HFS protocol consisting of 5 trains of pulses (200 Hz, 100 ms at a rate of 1 per s). This HFS protocol was presented 6 times in total, at intervals of 1 minute. The 50 \(\mu\)s, square, biphasic pulses used to evoke LTP were applied at the same intensity as for the single pulse presented following CS presentation (Gruart et al., 2006). Prior to the HFS session, baseline fEPSP records were recorded for 15 minutes. After HFS, fEPSP records were continued for 60 minutes fEPSPs were recorded for 15 minutes on 3 additional days following the HFS session (Fig. 6).

2.7. Histology

At the end of the experiments, mice were deeply reanesthetized (sodium pentobarbital, 50 mg/kg), and electrolytic marks were made at brain recording and stimulating sites (2 mA, 10 s). Animals were then perfused transcardially with saline and 4% phosphate-buffered paraformaldehyde. Se-
lected sections (50 μm) including the dorsal hippocampus and the frontal cortex were mounted on gelatinized glass slides, and stained using the Nissl technique (0.1% toluidine blue) to determine the location of stimulating and recording electrodes (Fig. 1D and E).

2.8. Data analysis

EMG and hippocampal activity, and 1-volt rectangular pulses corresponding to CS and US presentations, were stored digitally on a computer through an analog/digital converter (CED 1401 Plus, Cambridge Instruments, Cambridge, England), at a sampling frequency of 11–22 kHz and an amplitude resolution of 12 bits. Data were analyzed off-line for quantification of conditioned responses (CRs) with the help of the Signal Average Program (Cambridge Instruments, Cambridge, England, UK). We considered a response to be conditioned when the rectified EMG activity, during the CS-US period, presented the following conditions: (1) the EMG activity lasted > 10 ms; (2) the EMG was not preceded by any spontaneous activity in the 200 ms preceding CS presentation; (3) the EMG activity was initiated > 50 ms after CS onset; and (4) the integrated EMG activity was at least 2.5 times greater than the activity recorded 200 ms before CS presentation (Domínguez-del-Toro et al., 2004).

Commercial computer programs (Signal 3, from CED) were configured to represent EMG and fEPSP recordings. Data
were analyzed off-line for quantification of CRs and fEPSP amplitudes with the help of home-made representation programs (Domínguez-del-Toro et al., 2004; Gruart et al., 2006; Porras-García et al., 2005). Data were processed for statistical analysis using the SPSS for Windows package (16.0.2; SPSS Inc., Chicago, Il). Collected data were analyzed using analysis of variance (ANOVA), with time or session as repeated measure, and coupled with contrast analysis when appropriate. One-way ANOVA allowed checking the statistical differences between different groups.

3. Results

3.1. Functional properties of the eyelid motor system in SAMR1 and SAMP8 mice

As a preliminary control, we determined whether neural premotor eye blink circuits functioned similarly in SAMR1 and SAMP8 mice. Reflexively evoked eye blinks are usually characterized by the latency of their early (R1) and late (R2) components (Kugelberg, 1952) and by the quantification of their integrated EMG areas (Gruart et al., 2006). The absence of significant differences (p ≥ 0.93, Student t test) between reflex eye blinks...
in the 2 groups of animals allowed us to use the classical conditioning of eyelid responses to test their associative learning capabilities (see below).

### 3.2. Input/output relationships at the CA1-medial prefrontal synapse in SAMR1 and SAMP8 mice

In a second series of experiments, we checked the functional state of the CA1-medial prefrontal synapse. For this we studied the changes in the amplitude of fEPSPs evoked in the medial prefrontal cortex by paired-pulse (40 ms interstimulus interval) stimulation of the CA1 area. As illustrated in Fig. 2A for SAMR1 mice, the amplitude of fEPSPs (in mV) evoked in the medial prefrontal cortex by

the first pulse increased with current strength until reaching asymptotic values. In addition, fEPSPs evoked by the second pulse increased more-or-less in parallel with the fEPSPs evoked by the first pulse, but with larger values \( F(1,28) = 488.389; p < 0.001 \) for asterisks illustrated in Fig. 2A. In contrast to a recent description for input/output curves at the CA3–CA1 synapse (Madroñal et al., 2009), fEPSP amplitudes evoked by the second pulse were never significantly smaller than those evoked by the first. Similar displays were obtained for data collected from SAMP8 animals (Fig. 2B) — namely, the paired-pulse facilitation evoked at low stimulus intensities (<2.4 mA) was more evident \( F(1,22) = 189.451; p < 0.001 \) for asterisks illustrated in Fig. 2B.

Fig. 5. Representation of the attention devoted to a familiar (Fam) or a novel (New) object exhibited by SAMR1 (A) and SAMP8 (B) groups, during an object recognition task, for the training (0 hours) session, and 1, 24, and 72 hours afterward. The object presentation sequence is schematized at the bottom. Values are mean ± standard error of the mean (SEM) of the percentage of the total attention exhibited in each session. Statistical differences between percentages of attention: ** \( p < 0.01 \); *** \( p < 0.001 \) (1-way analysis of variance [ANOVA]).
the facilitation in response to higher stimulus intensities (>2.4 mA). Interestingly, input/output curves collected from SAMR1 animals presented significantly larger values for both the first and the second pulse than values collected from SAMP8 mice. F(1,28) = 4.84; p < 0.05 for first fEPSP and F(1,28) = 230.8; p < 0.001, for stimuli = 0.6 mA]. Nevertheless, no significant differences between the 2 groups of animals were observed for paired-pulse facilitation for pairs of stimuli of increasing intensity (Fig. 2C).

As a whole, these data suggested that, for a given time interval (40 ms), the facilitation evoked by paired-pulse stimulation was more noticeable at low intensities. Moreover, input/output curves evoked for this interval presented lower fEPSP amplitudes in SAMP8 animals than in controls.

Fig. 2D illustrates the result of checking the effect of selected intensity (~40% of the intensity necessary for evoking a maximum fEPSP response with the first pulse) applied at different interstimulus intervals (10, 20, 40, 100, 200, and 500 ms). Peak facilitation (>250%) was observed for an interstimulus interval of 40 ms in both groups of animals. Paired-pulse facilitation was also noticed at an interstimulus interval of 20 and 100 ms [F(5,84) = 50.06; p < 0.001 for SAMR1 and F(5,81) = 26.89; p < 0.001 for SAMP8]. No significant differences were observed for paired-pulse facilitation between the 2 experimental groups.

In an additional series of experiments, we checked the effects of paired-pulse stimulation at increasing intensities (0.3–4.8 mA) for a wide range of inter-stimulus intervals (10, 20, 40, 100, 200, and 500 ms). As illustrated in Fig. 3A and B, the CA1-medial prefrontal synapse presented a definite pattern of facilitation and depression to paired-pulse stimulation in SAMR1 animals, depending on the selected intensity and interstimulus interval. On the whole, SAMP8 animals (Fig. 3C and D) reached lower values than their controls in this tridimensional representation of input/output curves. These results suggest that in relation to neurotransmitter release at the CA1-medial prefrontal cortex synapse, the effects of action potentials arriving at CA1 axon terminals in SAMP8 animals were different from those evoked in SAMR1, mainly with regard to peak fEPSP amplitudes following the first and the second pulse. Interestingly, these significant differences in input/output curves did not affect paired-pulse facilitation.

3.3. Classical conditioning of eyelid responses in SAMR1 and SAMP8 mice

Fig. 4 illustrates some raw records (Fig. 4A and B) and the mean percentage of CRs (Fig. 4D) across habituation, conditioning, and extinction sessions for the 2 (SAMR1 and SAMP8) experimental groups.

During conditioning sessions (Fig. 4D, black circles), control animals displayed an acquisition curve characterized by a progressive increase in the percentage of CRs. These animals presented a mean percentage of 31.3 ± 2.5 (mean ± standard error of the mean [SEM], n = 6) CRs on the first conditioning day, and reached asymptotic values by the fourth conditioning session (44.7 ± 5.2%). In contrast, SAMP8 animals reached significantly lower values (20.2 ± 6.6%) during the first conditioning session and 17.5 ± 5.9% during the fifth, n = 7). Thus, SAMP8 animals seem incapable of acquiring this type of associative learning task. Differences in the percentage of CRs obtained by the 2 groups reached statistical significance from the third to the fifth conditioning [F(1,11) = 13.096; p < 0.01] and for the first extinction (p < 0.05) sessions (Fig. 4D).

3.4. Learning-dependent changes in strength at the CA1-medial prefrontal synapses during classical eye blink conditioning in SAMR1 and SAMP8 mice

Fig. 4C illustrates the evolution of fEPSPs evoked at the CA1-medial prefrontal cortex synapse across the successive habitation, conditioning, and extinction sessions. fEPSPs evoked in SAMR1 animals showed a sharp increase in amplitude (170.9%) during the first conditioning session as compared with baseline (100%) values (i.e., those collected during the habitation session). The amplitude of fEPSPs evoked at the CA1-medial prefrontal synapse was even larger during the fifth conditioning session (176.1%), but decreased during the 3 extinction sessions to almost baseline values. fEPSP amplitudes evoked during the 5 conditioning sessions (but not during the 3 extinction sessions) were significantly larger than baseline values in SAMR1 mice [F(5,50) = 4.23; p < 0.05]. In contrast,
fEPSPs evoked in SAMP8 animals across conditioning and extinction sessions presented similar amplitude values to those collected during baseline records \((p = 0.53)\). Moreover, the amplitudes of fEPSPs recorded from SAMR1 mice were significantly \(F(5,50) = 4.47; p < 0.05\) larger than those collected from SAMP8 animals during the conditioning sessions (see asterisks in Fig. 4C). Taken together, these results indicate that SAMP8 animals were both unable to acquire this type of associative learning task and to present a concomitant activity-dependent potentiation of the CA1-medial prefrontal cortex synapse.

3.5. Performance of the object recognition test by SAMR1 and SAMP8 mice

In another series of experiments, we determined the learning capabilities of SAMR1 and SAMP8 animals in an object recognition task. As illustrated in Fig. 5, during the acquisition period the 2 groups of animals spent similar amounts of time (about 50%) exploring 2 identical objects (O1 and O2), indicating no spatial preferences associated with their location. Because the total time of approach to the 2 objects varied considerably in the different animals, we preferred to use percentage of attention as a quantitative index. As indicated in 2. Methods, the percentage of attention was defined as the time spent exploring each object (familiar or new) divided by the time spent exploring both objects and multiplied by 100. The percentage of attention during the acquisition period was similar for objects O1 and O2 for both SAMR1 \(F(1,16) = 0.83; p = 0.77\) and SAMP8 animals \(F(1,16) = 0.023; p = 0.88\).

During the first choice trial, 1 hour after the initial training session, mice were allowed to explore a novel object (B1) and a familiar one (O3). In this case, the analysis of variance of the collected data indicated that SAMR1 mice presented a significant \(F(1,16) = 8.83; p < 0.01\) increase in the percentage of attention devoted to the novel object (Fig. 5, 1-hour session). In contrast, SAMP8 animals spent significantly \(F(1,16) = 15.6; p < 0.01\) more time with the familiar object than with the novel one.

During the second choice trial, carried out 24 hours after the acquisition period (Fig. 5, 24-hour session), animals were presented with a novel object (C1) and a familiar one (B2). In this case again, a significant \(F(1,16) = 26.89; p < 0.001\) increase in the percentage of attention spent exploring the novel object was found for SAMR1 animals. In contrast, the SAMP8 group failed to spend more time exploring the novel versus the familiar object \(F(1,16) = 0.028; p = 0.87\).

During the last choice trial, 72 hours after the training session (Fig. 5, 72-hour session), the mice were allowed to explore a novel object (D1) and a familiar one (C2). Here again, SAMR1 animals explored the novel object for a significantly \(F(1,16) = 61.93; p < 0.001\) longer period of time than the familiar one, while the SAMP8 group devoted more time to exploring the familiar object than the novel one, reaching significantly larger values \(F(1,16) = 39.33; p < 0.001\) in this task.

In summary, and as already described for classical eye blink conditioning, SAMP8 mice seemed incapable of properly performing the object recognition tasks, as the control group did. A parsimonious interpretation of collected results is that SAMP8 mice presented a sort of neophobia to the exploration of novel objects, concentrating their exploring activities on familiar ones. At this regard, gustatory and/or food neophobia have been reported as a typical sign of the aging process (Collier et al., 2004; Hsiao et al., 1995).

3.6. Differences in the LTP evoked at the CA1-medial prefrontal cortex synapse in SAMR1 and SAMP8 mice

In contrast with the evolution of LTP evoked in behaving mice at the CA3–CA1 synapse (Gruart et al., 2006), LTP evoked at the CA1-medial prefrontal cortex synapse in (SAMR1) controls presented a slow build-up and a not very large change in the amplitude of evoked fEPSPs (Fig. 6, black circles). Thus, the LTP evoked in SAMR1 mice reached values significantly larger (determined by the amplitude of evoked fEPSPs) than those collected during baseline recordings only 48 hours after the HFS session \([\sim 120\%, F(1,15) = 5.03; p < 0.05]\). Nevertheless, the LTP evoked in SAMP8 animals was still present, and significantly different from baseline values \((p < 0.01), 72\) hours after the HFS session. In contrast, SAMP8 animals did not present any significant change in the amplitude of evoked fEPSPs following the HFS session. In fact, a nonsignificant decrease in fEPSP amplitudes was observed 24 hours, 48 hours, and 72 hours after HFS. In summary, it can be concluded that LTP evoked at the CA1-medial prefrontal cortex synapse in (SAMR1) controls presents some interesting differences with LTP evoked in behaving mice at the CA3–CA1 synapse (Gruart et al., 2006). In addition, SAMP8 animals failed to produce any LTP at the CA1-medial prefrontal synapse following HFS.

4. Discussion

4.1. General remarks

We have shown here that SAMP8 mice are unable to acquire a classical eye blink conditioning using a hippocampal- and prefrontal-dependent trace paradigm (Clark and Squire, 1998; Jay et al., 1996; Moyer et al., 1990; Thompson and Krupa, 1994). In contrast, SAMR1 mice acquired this associative learning task with profiles and asymptotic scores similar to those previously reported in wild-type mice, using similar conditioning procedures (Domínguez-del-Toro et al., 2004; Gruart et al., 2006, 2008). The inability of SAMP8 mice to acquire this associative learning task cannot be ascribed to a deficit in the activation of the trigeminal receptor terminals, because experimentally evoked blink reflexes were performed equally in SAMP8 and SAMR1 animals. In addition, we have shown here that...
the hippocampal CA1-medial prefrontal pathway is activated in parallel with the acquisition process in control SAMR1, but not in SAMP8, mice, indicating a significant deficit in the storage of acquired memories in the latter (Jay et al., 1996; Squire and Alvarez, 1995). SAMP8 mice also have significant deficits in the acquisition of an object recognition test, another task requiring the proper functioning of hippocampal and prefrontal circuits (Baran et al., 2010; Clarke et al., 2010).

We have also shown that LTP, evoked by HFS of pyramidal CA1 projections to the medial prefrontal cortex, is impaired in alert behaving SAMP8 mice. Again, the functional impairment in evoking LTP cannot be ascribed to a complete deficit in the activation of the CA1-medial prefrontal synapse, because synaptic basal transmission was not affected in the least at the intensities used in this study — namely, about 1 third of the amount necessary for reaching asymptotic values in the input/output curves. Thus, the functional transformations taking place in hippocampal CA1-medial prefrontal pathways seem to be necessary for the proper acquisition of CRs, and this process is severely impaired in SAMP8 mice, in comparison with their (SAMR1) controls.

4.2. Associative learning capabilities of SAMR1 and SAMP8 mice

Classical eye blink conditioning is a widely used technique (Gruart et al., 1995; Koekkoek et al., 2002; Marshall-Goodell et al., 1992; Thompson and Krupa, 1994; Weiss et al., 1999) that appears to be a very useful tool for comparative studies both in genetically manipulated animal models (Domínguez-del-Toro et al., 2004; Ewers et al., 2006; Gruart et al., 2008; Takatsuki et al., 2003; Weiss et al., 2002) and in natural aging and neurodegenerative diseases (Thompson et al., 1996; Woodruff-Pak et al., 1990). In addition, the acquisition of trace conditioning paradigms is highly related to the proper functioning of hippocampal circuits (Gruart et al., 2008; Moyer et al., 2000; Takatsuki et al., 2003), while its proper storage in the form of stable memories is dependent on the adequate transfer of information from the hippocampus to the prefrontal cortex (Clark et al., 2000; Jay et al., 1996; Lopes Aguiar et al., 2008; Squire and Alvarez, 1995).

According to the present results, evident functional alterations in the acquisition and storage of a trace conditioning paradigm are present in SAMP8 mice. Interestingly, SAMP8 animals used here presented these associative learning deficits at earlier ages (5–6 months old), as compared with the case of similar deficiencies observed in different types of transgenic mice (7–18 months old) modeling Alzheimer’s disease (Domínguez-del-Toro et al., 2004; Gruart et al., 2008; Lee et al., 2004). Because there is no evidence of the presence of amyloid β deposits or of neurofibrillary tangles in SAMP8 mice of the age used here (see Takeda, 2009 for references), it seems clear that factors besides plaque deposits are involved in the functional deficits observed in this type of senescence-accelerated animal (Blanchard et al., 2003; Selkoe, 2002). For example, 5-month-old SAMP8 brain suffers hyperphosphorylation of microtubule-associated protein tau and increased cytosol and mitochondrial oxidative stress, leading to a mitochondrial membrane fluidity reduction and adenosine-5'-triphosphate (ATP) deficit (Alvarez-García et al., 2006; Canudas et al., 2005; Carretero et al., 2009; García et al., 2010) that may cause changes leading to abnormal brain circuit function. On the other hand, the functional modifications in the hippocampal-medial prefrontal pathway could be ascribed to the early affectation of cholinergic terminals projecting to cortical structures (Fernández de Sevilla et al., 2002). Indeed, 6- and 8-month-old SAMP8 mice present brain cholinergic deficits that parallel the severity of cognitive impairment (Matsui et al., 2009; Wang et al., 2009).

4.3. Activity-dependent plasticity in the pyramidal CA1-medial prefrontal synapse during associative learning in SAMR1 and SAMP8 mice

The involvement of hippocampal unitary activity in the acquisition of classical eye blink conditioning has been convincingly shown in rabbits (Berger et al., 1983; McEchron and Disterhoft, 1997) and cats (Münera et al., 2001). Indeed, it has been shown that hippocampal pyramidal CA3 and CA1 firing to CS presentation increases well in advance of the appearance of the CR (McEchron and Disterhoft, 1997; Münera et al., 2001). Moreover, it has recently been shown in behaving mice that the slope of fEPSPs evoked at the CA3–CA1 synapse increases across sessions in a trace conditioning paradigm (Gruart et al., 2006), a fact that supports the increased firing in the CA1 area during acquisition of associative learning tasks (McEchron et al., 2003; Münera et al., 2001). At the same time, both medial and orbital prefrontal cortices are involved in the emotional component of selective behaviors, including classical and instrumental conditionings (Corbit and Balleine, 2003; Powell et al., 1996). In particular, the medial prefrontal cortex seems to participate in stimulus salience, sustained attention (Weible et al., 2003), and/or the integration of learned emotional changes (Powell et al., 1996). Specifically, it has been shown that the lesion of the caudal part of the medial prefrontal cortex impairs the acquisition of a trace conditioning task in rabbits (Harvey et al., 2004; Kronforst-Collins and Disterhoft, 1998). A recent study in behaving rabbits has shown that the medial prefrontal cortex is of crucial importance in establishing the emotional (aversive) components of trace eye blink conditioning (Leal-Campanario et al., 2007). Finally, it has already been reported that the CA1-medial prefrontal synapse is involved in cognitive processes such as learning and memory, and is susceptible to modification by HFS protocols (Izaki et al., 2003; Jay et al., 1996; Lopes Aguiar et al., 2008).
Here we are reporting an activity-dependent potentiation of the pyramidal CA1-medial prefrontal cortex synapse across the successive conditioning session, but only for SAMR1 mice. SAMP8 mice failed to show any sign of synaptic potentiation, in parallel with their inability to acquire a significant amount of CRs. Similar deficits (for both acquisition of CRs and for hippocampal synaptic plasticity) have already been reported in aged (18-month-old) wild-type mice (Gruart et al., 2008). Thus, both normal aging (Gruart et al., 2008) and accelerated senescence (the present report) seem to be processes sufficient to impair both associative learning and the underlying changes in synaptic plasticity. Another possible interpretation of the present results is that SAMP8 mice present some developmental deficits producing the reported results, although this fact is not completely supported for previous studies of the same animals at different ages (Hosokawa et al., 1984; Zheng et al., 2009).

4.4. Other behavioral deficits observed in SAMP8 mice

It has already been reported that, compared with SAMR1 animals, SAMP8 mice present significant deficits in passive and active avoidance tasks (Miyamoto et al., 1986) and spatial learning (Miyamoto, 1997), but there is not much information regarding SAMP8 performance during object-recognition tests (Fontán-Lozano et al., 2008). Because evidence suggests that hippocampal-prefrontal circuits is one of the early traits of cognitive decline observed in Alzheimer’s patients (Baran et al., 2010; Clark et al., 2000, 2010; Grady et al., 2001), we checked here how the 2 groups of animals performed this simple cognitive task. Results obtained with the object recognition test suggest that SAMP8 mice presented a preference for attending familiar objects and/or and associated difficulty for exploring novel objects. As already reported in rats with electrolytic lesions in the medial prefrontal cortex (Baran et al., 2010), SAMP8 mice showed a tendency to overexplore familiar objects instead of exploring new ones, i.e., a sort of neophobic syndrome (Fontán-Lozano et al., 2008). Indeed, gustatory and/or food neophobia has been already reported as a determining sign of the aging process (Collier et al., 2004; Hsiao et al., 1995). Thus, the object recognition test used here further confirms the affectation of hippocampal and prefrontal circuits by the accelerated senescence present in SAMP8 mice (Takeda, 2009).

4.5. Differing characteristics of LTP evoked at the CA1-medial prefrontal synapse in SAMR1 and SAMP8 mice

Most available information regarding LTP evoked at the CA1-medial prefrontal synapse was collected from slices or from anesthetized animals (Izaki et al., 2003; Lim et al., 2010; Lopes Aguiar et al., 2008) and cannot be easily compared with the study over time carried out here. According to the present results, LTP evoked at the CA1-medial prefrontal cortex synapse in SAMR1 mice builds up slowly, reaching peak values 2–3 days after the HFS session. This pattern is characteristically different from previous descriptions of LTP induction at the CA3–CA1 synapse in alert behaving mice (Gruart et al., 2006) and is suggestive of a slow processing of information transferred from the hippocampus to the prefrontal cortex related to late consolidation of acquired memories. It has been reported recently that muscarinic acetylcholine neurotransmission may enhance late phases of LTP developed in the hippocampus-prefrontal cortex pathway. Although LTP evoked at the synapse between CA1 and subiculum to the prefrontal cortex in the rat did not present such a delayed LTP, an activity-dependent increase in strength started to develop during overtraining in a classical conditioning paradigm (Jay et al., 1996). Interestingly, SAMP8 mice did not show any significant increase in fEPSP amplitude following HFS, a fact that further confirms their inability to present activity-dependent changes in synaptic strength during an associative learning task. Similar deficits for evoking LTP at the dentate gyrus-CA3 and at the CA3–CA1 synapses have already been described in SAMP8, using in vitro procedures (Katsuki et al., 1990; Yang et al., 2005), suggesting that the synaptic deficits reported here are extensible to other hippocampal circuits. In summary, SAMP8 animals could be devoid of the neural mechanism involved in the late consolidation of learned associations (Jay et al., 1996; Squire and Alvarez, 1995).

Disclosure statement

The authors do not have any actual or potential conflicts of interest.

All the experiments were carried out according to European Union Council (2003/65/EU) and Spanish (BOE 252/34367-91, 2005) guidelines for the use of laboratory animals in chronic electrophysiological and behavioral studies. All experimental protocols were also approved by the Ethics Committee of the Pablo de Olavide University.

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Uncited References

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further instruction for this reference, we will retain it in its current form and publish it as an “un-cited reference” with your article. *Wong et al., 1999.*

**References**


